Size Matters

Size Exclusion Chromatography and UHPLC // Quick and straightforward, aqueous size exclusion chromatography (SEC) has become a mainstay for the analysis of protein aggregates in the biopharmaceutical industry. Coupling SEC with advanced detection, such as mass spectrometry, light scattering or surface plasmon resonance, makes it a versatile tool for numerous applications beyond aggregate analysis.

LAB Tip+

more information:

- You can find more information about **SEC** on our homepage www.lab-worldwide.com.
- Visit Tosoh Bioscience at the **Ilmac Lausanne** from October 3 to 4 in Lausanne/Switzerland (Stand 7/A01).

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The antibody therapeutics market is enjoying high growth rates, the major areas of therapeutic application being cancer and immune/inflammation-related disorders including arthritis and multiple sclerosis. In 2017, five of the top ten best-selling global drug brands were monoclonal antibodies (mAbs) and more than 400 mAbs or mAb related drugs were in clinical trials. The characterization and monitoring of these complex biomolecules is a major challenge throughout the entire development and manufacturing process.

Timelines in the analytical laboratories are increasingly tough. On the other hand, there is a strong drive to explore and understand biologics more in detail. With new biopharmaceutical formats in the pipeline — bispecific antibodies and antibody-drug conjugates, for example — rapid and thorough characterization will be even more important. In this context, the task for separation science is to increase resolution and plate numbers in shorter analysis time. Applying latest UHPLC column technology improves the quality of separation and thereby the reliability of results.

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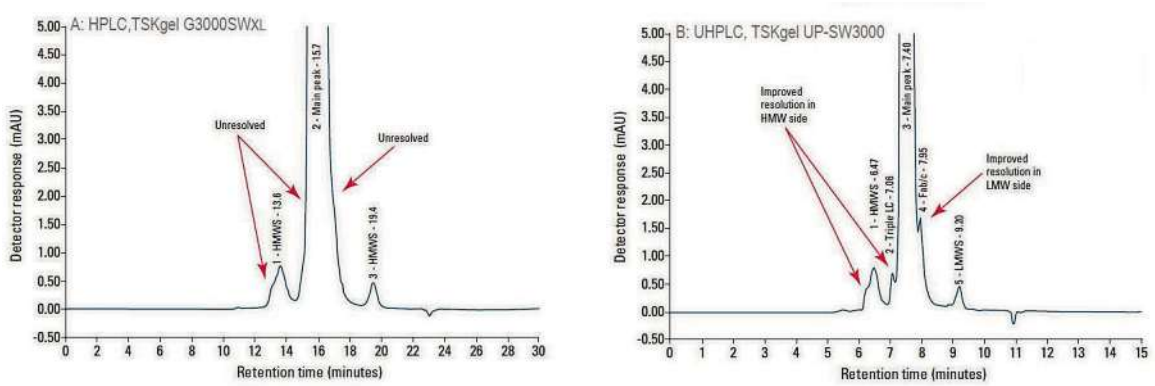
Antibody Aggregate Analysis

The separation of mAb monomers from their aggregates is one of the standard assays at all stages of mAb development and manufacturing. Size exclusion chromatography is

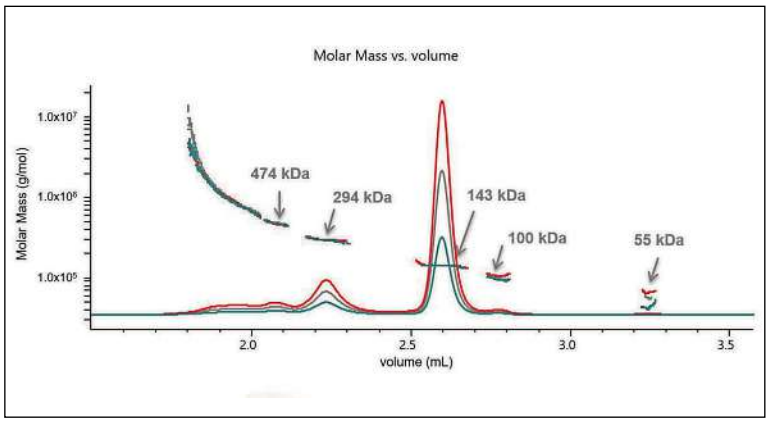
Sources: Tosoh Bioscience



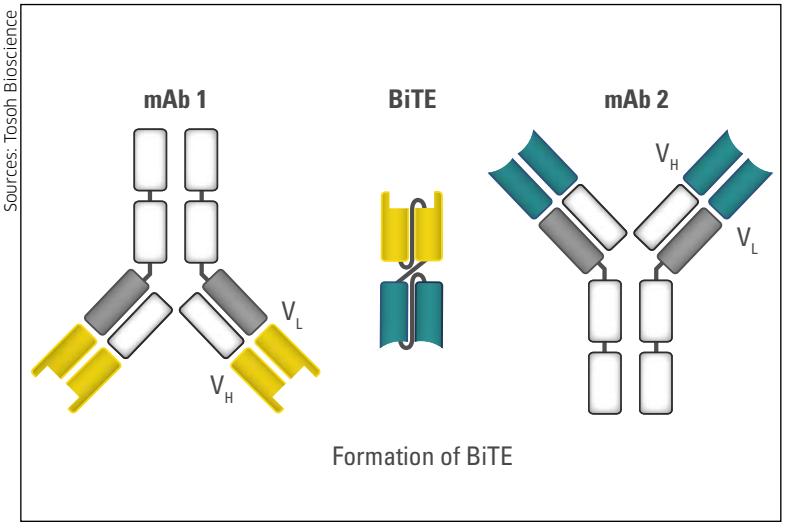
2 Comparison of mAb aggregate analysis using HPLC (TSKgel G3000SWXL) and UHPLC (UP-SW3000) methods.

the method of choice for these types of analyses. UHPLC (ultra-high performance liquid chromatography) is already a standard technology in the analysis of small-molecule drugs so it is no surprise that biopharmaceutical manufacturers are now looking to follow suit. For decades, The Tosoh G3000SWXL has been the gold standard for aggregate detection in the biopharmaceutical industry. Featuring the same pore size and surface chemistry but smaller particle size, The UP-SW3000 is the UHPLC column of choice when transferring established HP-SEC methods to UHP-SEC.

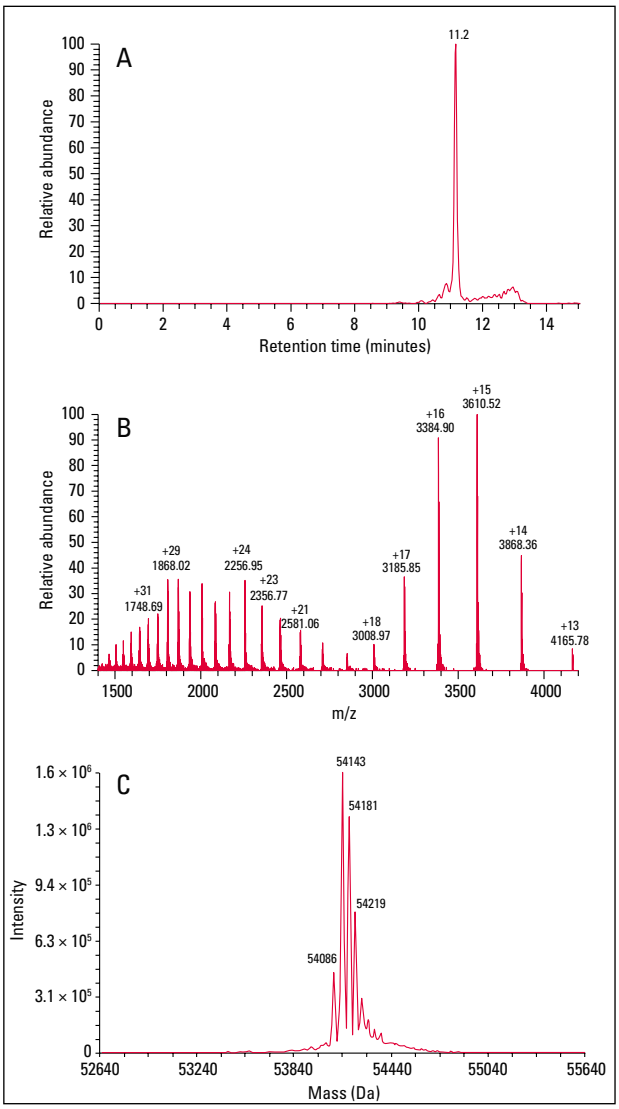
Figure 1 demonstrates the advantages of the the column (4.6 mm x 30 cm, 2 µm, 250 Å, flow rate 0.35 mL/min) used with a UHPLC



3 Analysis of a therapeutic antibody with UHP-SEC-MALS (three different injection volumes, Wyatt µDawn Detector). Data provided by courtesy of D. Roessner, Wyatt Technology Europe



4 Formation of Bispecific T Cell Engager



5 SEC/MS analysis of a Bite (performed by the Wistar Proteomics and Metabolomics Facility, Philadelphia, PA) a) total ion chromatogram, (b) mass spectrum and (c) deconvoluted mass spectrum

system for mAb analysis versus the use of a TSKgel G3000SWXL column (7.8 mm x 30 cm, 5 µm, 250 Å, flow rate 0.5 mL/min) with a conventional HPLC system. It offers higher resolution of both the high molecular weight species and the fragments. In addition, the analysis was completed in half the run time.

When integrating a new UHPLC column in routine analysis at big pharma, a thorough evaluation takes place. Important features besides the pure chromatographic performance are life time studies and lot-to-lot reproducibility. Both are known to be critical for many commercial UHP-SEC columns. In customer evaluations, the UP-SW3000 column performed extraordinary well. Lifetime was reported to be high, even with demanding new antibody formats, and lot-to-lot reproducibility is excellent.

UHP-SEC and Light Scattering

Coupling SEC with multi angle light scattering (MALS) detection enables absolute quantification of a molecule's molar mass, irrespective of elution time.

SEC-MALS has become a valuable tool for verifying purity of monoclonal antibodies as such or as a quick check while the downstream processing takes place. Besides fluorescence detection, light scattering is one of the most sensitive methods to detect protein aggregates.

The quality of SEC analysis is always dependent on a low extra column dead volume of the LC system used. Hence, transferring SEC-methods to UHPLC also requires special attention to the instrumentation besides the selection of the proper UHPLC column. While all modern UHPLC systems provide extremely low dead volumes and small detector cell volumes for UV detection, this is more complicated when it comes to MALS detection.

The Wyatt µ-Dawn, a three-angular MALS detector, is ideally suited for UHP-SEC and was used with the UP-SW3000 column to analyze the aggregates of a therapeutic antibody (Fig. 3).

A comparison of the MALS chromatogram with the UV and refractive index signals (data not shown) revealed that the small peak

achieved by the UHPLC column was retained for all three detectors.

UHP-SEC-MS for Antibodies

More potent antibody formats, such as bispecific antibodies (bsAbs), are on the rise. Bispecific antibodies recognize two different epitopes. This dual specificity increases the potency of these molecules compared to mAbs and expands the range of possible applications. More than 50 bsAb products are currently undergoing clinical evaluation. Characterization of bsAbs is essential to ensuring product safety and efficacy. Size exclusion chromatography (SEC) coupled with mass spectrometry (MS) is increasingly being used to identify the accurate molecular mass of biomolecules. SEC-MS, however, requires the use of volatile mobile phases and the use of columns that do not exhibit particle shedding which will interfere with the MS signal.

A bispecific T cell engager consisting of two single-chain variable fragments (scFvs) recombinantly linked by a nonimmunogenic five-amino-acid chain (Fig. 4) was analyzed by SEC-MS using a TSKgel UP-SW3000, 2 µm column. Figure 5 shows the total ion chromatogram, mass spectrum and deconvoluted mass spectrum of the Bite. A main peak can be seen at m/z 54,143; adjacent peaks correspond to different salt adducts.

These results demonstrate accurate molar mass determination utilizing a ammonium acetate/ammonium bicarbonate mobile phase with SEC-MS compatibility. Blank injections before and between each analysis proved the absence of shedding or carryover (data not shown).

Summary

Today, UHPLC is increasingly used for the analysis of biomolecules. Considering the complexity of large proteins such as antibodies, the transfer from established HPLC methods to UHPLC is not as easy as for small molecules.

For size exclusion chromatography, the new UP-SW3000 UHPLC column from Tosoh Bioscience offers an easy and straightforward method transfer to UHPLC. At a smaller particle size it features the same pore size and surface modification as the well-stablished G3000SWXL HPLC column. Its high durability and good batch-to-batch reproducibility are the main reasons for its rising popularity in the biopharmaceutical industry.

The easy implementation in SEC-MS and SEC-MALS methods is an additional benefit when it comes to the characterization of new biopharmaceutical formats such as bispecific antibodies, antibody-drug conjugates or virus like particles. ■